Evaluation of gastric and duodenal antiulcer activity of ranitidine formulation in experimental animals

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ABSTRACT
The present study involves the evaluation of controlled release formulation of Ranitidine for its gastric and duodenal antiulcer activity in rats as animal models. Gastric and duodenal ulcers were produced in rats by pyloric ligation method as described by Shay et al.[1] and aspirin induced ulcer in rats. The animals were divided separately for both experiments. In each method animals were divided into four groups of six animals each. Group 1 served as normal control in which the animals received only distilled water. Group II served as disease control in which the animals were maintained under same environmental conditions but surgical manipulations done like other groups. Group III received standard drug Ranitidine 50mg/kg orally[2]. Group IV were received Ranitidine formulation respectively with a dose equivalent to Ranitidine 50mg/kg orally by means of suspension. The antiulcer activity of pyloric ligated and aspirin induced animals were correlated for the reduction in ulcer levels. Various parameters[3] like mean volume of gastric secretion, mean pH, mean total acid and ulcer index were calculated and was concluded that the group received Ranitidine formulation exhibited significant antiulcer activity by both methods when compared to standard drug Ranitidine. The biopsy report of rat stomach of all the groups were analyzed and was found that rats which received Ranitidine formulation and standard Ranitidine showed good healing of ulcers when compared to disease control group of animals. The mean volume of gastric secretions, mean pH mean total acid and ulcer index for Ranitidine formulation treated group was calculated as 2.67ml, 5.59, 110mEq/l, and 1.74 respectively. From the results it can be concluded that Ranitidine formulation exhibited significant antiulcer effect and the histopathology report also supports and confirm its effect.

INTRODUCTION
Approximately one third of the population in the society experiences regular dyspepsia, more than 50% of affected patients self medicate them self using OTC antacids and do not undergo medical advice. Most of
these patients suffer from gastro esophageal reflux or peptic ulceration. Zollinger–Ellison syndrome is induced by gastrin secreting tumours [4]. Gastric acid is secreted by the parietal cells in the gastric mucosa. The basolateral membrane region of these cells contains receptors for the three important chemical messengers of acid secretion, namely gastrin (from antral G cells), histamine (from entero chromaffin like cells) and acetyl choline (from vagal efferents). The gastric acid secreted by these chemical messengers and also by other factors like drug induced (NSAID’S) and also infection caused by *Helicobacter pylori* are mainly balanced or suppressed by certain protective agents that are produced by body’s defence mechanism. They are protectives like prostaglandins (PG’S), Mucus, Bicarbonate and mucosal blood flow [5]. Exogenous aggressive factors like smoke, anti-inflammatory drugs, alcohol, stress, fatty foods and helicobacter pylori infections triggered tissue necrosis through mucosal ischemia, free radical generation and cessation of nutrient delivery, hydrochloric acid together with pepsin, pancreatic enzymes and bile decreases the defence mechanisms of gastrointestinal mucosa such as the intercellular junctions, local blood flow, mucus/bicarbonate secretion and cellular growth and may also cause ulcer [6,7]. In recent years large advance in chemical and pharmacological studies has contributed to the knowledge about new therapeutically active compounds and controlled drug delivery systems for peptic ulcers.

Ranitidine is a H₂ receptor antagonist that inhibits acid production by reversibly competing with histamine for binding with H₂ receptors that is located at the basolateral membrane of the parietal cells [8]. H₂ receptor antagonists not only inhibit acid secretion induced by histamine, gastrin and cholinergic stimulation, they also promote healing of the duodenal ulcers [9]. Theoretical bio availability of Ranitidine is 50% [10] and the therapeutic dose levels are maintained for 6-8 hrs and very small amount of Ranitidine binds to proteins that require repeated dose administration [8] and it leads to increased adverse effect. In order to overcome these problems an attempt was made to prepare a controlled drug delivery system for Ranitidine and its pathological influence on stomach was studied.

**MATERIALS AND METHODS**

Ranitidine was procured as a gift sample from Novartis, Bombay. Topfers reagent and sodium hydroxide were procured from Merck, Mumbai. Wistar albino rats of either sex weighing 150-175gms were procured from National Institute of Nutrition and Science (NINS), Hyderabad.

**Pyloric ligation**

Wistar albino rats of both sex were grouped into eight each containing 6 animals. They were kept in the animal house at room temperature 25±2°C, with relative humidity of 45-55% maintained under 12hrs light and dark cycle and were fed with standard rat feed and were acclimatized for a week before the study [11,12]. Group I served as normal control in which distilled water was administered orally in which no pyloric ligation was done, group II served as disease control, group III received Ranitidine 50mg/kg orally and it was considered as standard, group IV served as Ranitidine Formulation group and the dose equivalent to Ranitidine 50mg/kg was administered.

Pyloric ligation was performed for Group II, III, and IV as described by Shay et al. Rats were fasted for 36hrs prior to the surgical procedure and kept in raised mesh-bottomed cages to avoid coprophagy. Under ether anesthesia the abdomen was opened by a
The pyloric portion of the stomach was identified, slightly lifted, avoiding traction to the pylorus or damage to the blood supply. The stomach was then replaced carefully and the abdominal wall closed by interrupted sutures. Animals were deprived of both food and water during the post operative period and were sacrificed at the end of 19-20hrs after the operation. The stomach was dissected out as a whole by passing a ligature at the esophageal end.

The stomach was separated from the surrounding tissues and organs and thus brought out as a whole along with its contents. The contents were subjected to centrifugation (3000rpm for 10mins) and then analyzed for mean volume of gastric secretion, mean pH and mean total acid. The pH was estimated by using indikrom pH strips (Glaxo India Limited, India) with pH ranges of 2-4.5 and 5-8.5 with a difference range of 0.5. Free acidity and total acidity were estimated by titrating 1ml of centrifuged sample with 0.01N NaOH, using Topfers reagent as indicator and phenolphthalein indicator respectively. Acidity was expressed in clinical units that are the amount of 0.01N NaOH base required to titrate 100ml of gastric secretion[13].

Acidity was expressed as:

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\text{Acidity} = \frac{\text{Volume of } \text{NaOH} \times \text{normality} \times 100}{0.1} \text{mEq/1}
\]

**Aspirin induced ulcer**

In Aspirin induced ulcer models[14] four groups of albino rats of either sex weighing 150-175g, with each group consisting of six animals were used. The first group served as a normal control the second group served as disease control and the third group served as standard group that received Ranitidine 50mg/kg and group four received Ranitidine formulation equivalent to Ranitidine 50mg/kg. All the animals received above treatment once daily for eight days orally. After 8days of treatment, animals were fasted for 24hrs. Ulcer was produced by administration of aqueous suspension of aspirin (200mg/kg orally) on the day of sacrifice. The animals were sacrificed 4h later and stomach was opened to calculate the ulcer index by kunchandy method[15].

The antulcer activity was carried out after the ethical approval from CPCSEA and it was done as per the recommended guidelines of CPCSEA reg. no- 1069/AC/07/CPCSEA).

**RESULTS AND DISCUSSIONS**

In aspirin and pylorus ligation induced gastric ulcer models the Ranitidine formulation reduced the gastric volume, total acidity and ulcer index (TABLE 1) thus showing the anti secretory mechanism involved in the antulcerogenic activity[2] through $H_2$ receptors.

Ulcer index parameter (TABLE 2) was used for the evaluation of antulcer activity since ulcer formation is directly related to the factors such as gastric volume, and total acidity[16]. From the results it is clear that gastric volume, pH, total acidity and ulcer index of formulated Ranitidine were significantly reduced as 2.67ml, 5.59, 110mEq/l, and 1.74 respectively.

The biopsy reports of all the groups of rats were analyzed and shown in (Figure 1a-d) and it was found that the section of stomach from normal control rat showed normal architecture, section of stomach from disease control rat showed severely damaged stomach cells with chronic inflammation, section of stomach from Ranitidine treated rat showed mild damaged cells and the section of Ranitidine formulation treated also showed mild damaged cells confirming the antulcer effect of Ranitidine formulation.
Formulated Ranitidine, and also there is no evidence of extra tissue damage as seen in the biopsy report. Hence it can be concluded that the formulated Ranitidine preparation could be used as a potential antiulcer agent for the treatment of duodenal and gastric ulcers.

REFERENCES